

Part IV Bioelectronics

Section 1 Genetic Analysis

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Chapter 1 Genosensor Technology Development

Chapter 1. Genosensor Technology Development

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1.1 Microdetection Technology for Automated DNA Sequencing

Sponsor

Genometrix, Inc.
Contract GMX-GH00776-04

Project Staff

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The overall objective of this cooperative work with Genometrix is to develop a novel method for automated, low-cost, high-throughput DNA sequence analysis. The overall goal is to demonstrate laboratory prototypes that provide a substantial increase in speed over the conventional DNA sequencing methods now used in the biomedical, pharmaceutical, and agricultural industries.

The basic approach being taken is depicted in figure 1. In a hypothetical DNA sequencing test, a solution of single-stranded "target" DNA strands of identical but unknown sequence is washed onto a specialized microelectronic chip called a genosensor. The genosensor surface contains a large array of test sites, each site containing short pieces of single-stranded DNA known as "probes." These probes are chemically attached to the site. All probes in a given site are of like sequence, and the sequence for each site is unique on the chip. The target DNA strands will bond, or hybridize, very strongly to probes containing their exact Watson-Crick complement, but much less strongly to probes on other sites. The sites containing hybridized DNA are identified via electronic sensing on the chip, and this information is used by off-chip instrumentation to reconstruct the sequence of the target strands.

MIT's role in this effort is to perform the design and fabrication of the genosensor chips.

The simplest electrical measurement that can be made at a test site to detect hybridization is probably a measurement of the change in local permittivity due to the addition of long target strands to the site. The complex permittivity $\epsilon' - j\epsilon''$ of an aqueous solution containing DNA exhibits a dispersion around a relaxation frequency which is a function of the size and conformation of the DNA molecule. A measurement of the capacitance and/or conductance between two electrodes in the solution over a range of frequency can therefore differentiate between a site that contains only short probe strands and one that contains long target strands hybridized to the probe strands. From these measurements the relative permittivity ϵ' , the dielectric loss ϵ'' and the dissipation factor ϵ''/ϵ' can be obtained for the cell.

The ideal electrode structure in a test well consists of two parallel plates spaced so that the entire volume between them is filled by the hybridized DNA globules in aqueous solution. For the sizes of target DNA envisioned, this spacing ranges from approximately 200 to a few thousand angstroms. A practical, easily fabricated structure that approximates this ideal is the interdigitated design shown in figure 2. Fabricated by a combination of wet and dry etching with metal liftoff, this design can achieve the required spacings between the upper and lower electrodes at their edges. Devices having either Au or Pt electrodes have been fabricated, with Pt preferred for most applications. Figure 3 shows various aspects of completed genosensor devices.

The primary emphasis of our work this past year was to optimize the electrode geometry for

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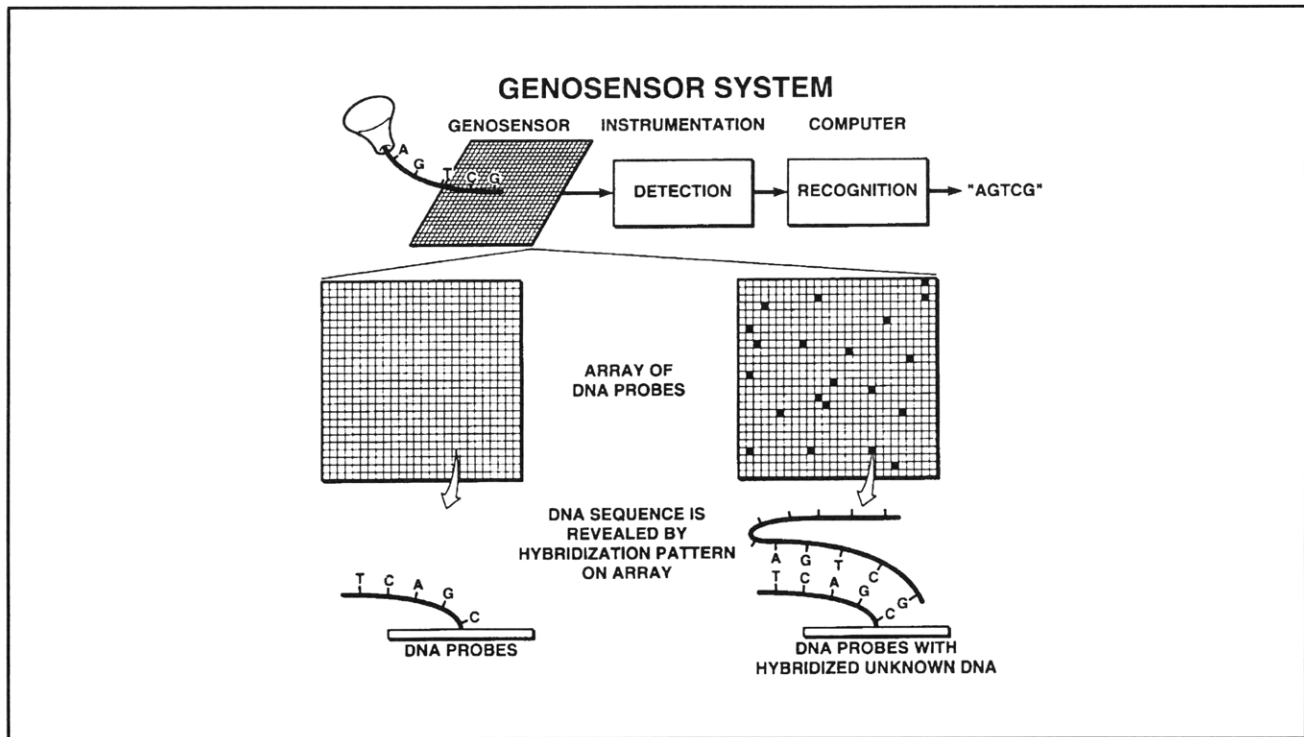


Figure 1. Conceptual genosensor system.

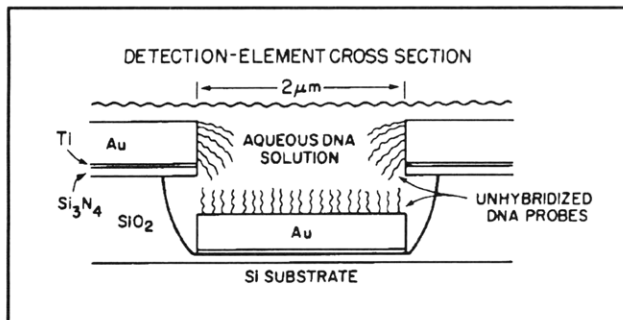


Figure 2. Electrode design for a permittivity genosensor. The unit cell shown is repeated many times across a test well to form an interdigitated test structure with the top Au electrodes connected to one access line and the bottom Au electrodes to the other.

maximum sensitivity. Initial work for this contract had begun with the electrode configurations illustrated in figures 2 and 3. Subsequently, both experimental studies and theoretical modelling showed a need to design and fabricate more aggressive electrode geometries. These have now been developed, as described below.

We have now successfully developed the technology for an aggressive self-aligned or split-architecture design using the same Ti-Pt electrode materials as in previous conventional genosensor designs. These new devices feature submicron-electrode spacings in both the vertical and horizontal dimensions. The patterning of the electrodes

is being done by a combination of laser interferometric lithography and reactive ion etching. A scanning electron micrograph of one of these devices is shown in figure 4. The electrode linewidth and spacing is 160 nm and the vertical separation of the interdigitated electrodes is approximately 200 nm. This geometry yields a 12.5 times improvement in the electrode density per unit device area. The bodies of the fingers themselves are Si₃N₄ to a depth of 200 nm, where thermal oxide is exposed. The use of oxide at the finger bottoms enables a slight undercut to be obtained by wet etching of the underlying oxide. It is anticipated that linker chemistries, designed to attach either to the closely-packed Pt electrodes or to the supporting dielectric posts, will enable a substantial increase in probe density at the sensor site, with a corresponding increase in detection sensitivity.

The development of this submicron self-aligned genosensor was enabled by successful resolution of three significant processing challenges: (1) excessive interelectrode metal shorts, (2) high interconnect-lead resistance, and (3) adaptation of our insulated-lead process to devices with submicron interelectrode spacings.

For the conventional 2-micron self-aligned genosensor geometries (shown in figures 2 and 3c), interelectrode shorts (connecting top and bottom) have been relatively rare due to the clean separation of metals resulting from the extensive oxide

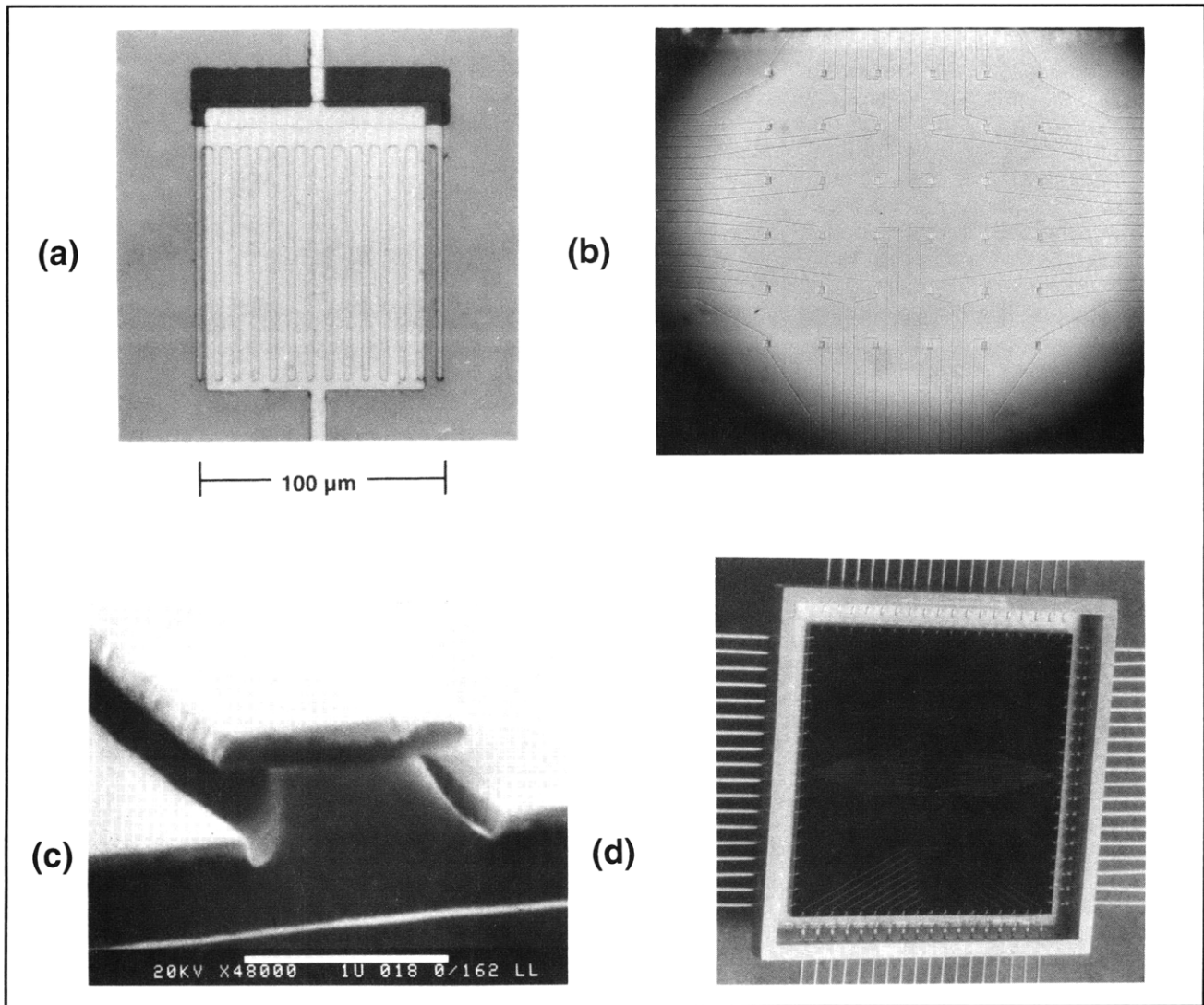


Figure 3. Collection of photographs showing various views of a complete genosensor device. (a) Top view of the standard $100\ \mu\text{m} \times 100\ \mu\text{m}$ single test cell. (b) Top view of the 6 X 6 passive array of test cells. (c) Scanning electron micrograph (SEM) closeup of the interdigitated electrode fingers. (d) Genosensor chip in electronic package.

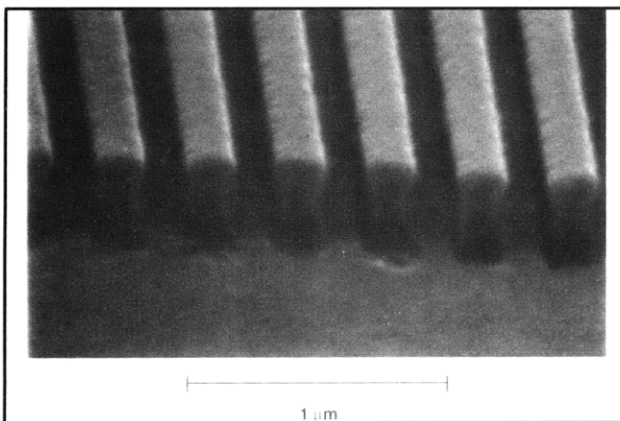


Figure 4. New submicron-periodicity genosensor structure.

undercutting inherent in the design. For the submicron-based devices, interelectrode shorts have been the leading cause of poor device yield. Because the grating etch is anisotropic and produces rather straight sidewalls, the sidewalls can end up being coated with a very thin metal layer after electron-beam deposition of the electrodes, resulting in an interelectrode short. From a measurement-sensitivity point of view, one would prefer to have the electrode layers relatively thick with minimal vertical and horizontal separation; however, this exacerbates the interelectrode shorting problem. Thus device yield and device sensitivity represent a fundamental tradeoff in device design and fabrication. We have encountered this problem in an array of other electronic devices which employ the same type of grating architecture. Two solutions to this problem that

have been successfully employed in the past are the use of controlled wet-chemical "dip" etches to remove the thin sidewall metal while leaving most of the active electrode intact, and/or the use of electrical probing to pass small amounts of current through the short to blow open the shorting link. Both techniques have their advantages and disadvantages. The primary advantage of the electrical unshorting method is that it can also serve as a diagnostic (in some instances) to indicate where the shorting problems are occurring. It should also be pointed out that the device remains functional provided most of the other grating fingers remain intact. In order to prevent the shorts from occurring to begin with, we have improved the process yield by two process alterations: (1) by increasing the amount of oxide undercut, and (2) by the use of shallow glancing-angle metal evaporations which are used to form a metal cap on the grating-finger tops. The net result of these two process improvements is illustrated by the SEM shown in figure 5. Note how the metal cap on the finger tops and the enhanced oxide undercutting serve to enhance the separation of the top and bottom metal electrodes. Typical short-related active-site failures have been reduced from 90 percent to approximately 10-15 percent with these process improvements, and the few remaining shorted devices are more easily fixed by nondestructive electrical probing.

Typical electrode metal thicknesses for the conventional-geometry genosensors were approximately 2000-2500 Å in thickness. Thus, the Pt-based interconnect lines were also of the same thickness. However, the submicron-period devices necessitate the use of 500-700 Å-thick metal electrodes for the device active areas. Thus, an additional metallization step is required for these devices in order to minimize the interconnect-lead resistance. The liftoff of thick Pt lines for the device interconnects is nontrivial, as has been detailed in past reports, but previous process development has enabled us to easily adapt this additional process with little or no consequence to device yield.

A somewhat more difficult task was the incorporation of the insulated-lead process developed originally for the conventional-geometry devices to eliminate spurious interconnect-associated signals. As described in previous reports, a process was developed to cover the long Pt interconnect lines with SiO₂, with subsequent exposure of the device active electrodes done by wet etching of the oxide in windows. This process was enabled in part by the large finger geometries of the conventional

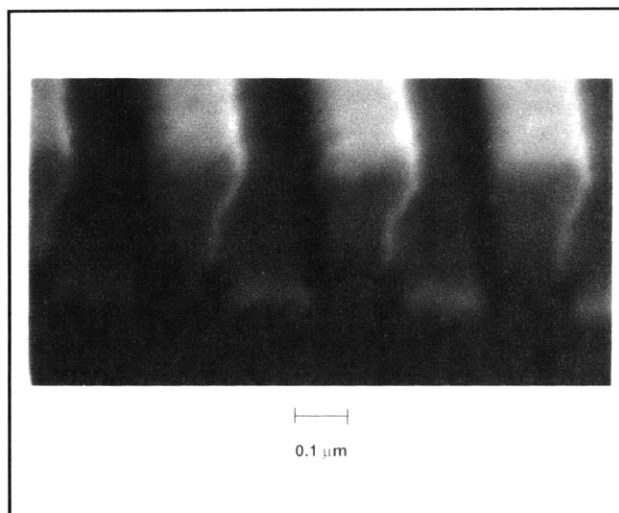


Figure 5. Closeup of new submicron-periodicity genosensor structure showing enhanced undercutting for minimization of metal-metal shorts.

devices which permitted some modest amount of overetching. It is clear, however, from the submicron-period device shown in figure 5 (note the very thin bottoms of the grating-finger posts which are SiO₂) that any overetch will likely result in either the complete collapse of the grating fingers or lifting of the metal-electrode lines off the surface. During this past year, we developed a two-step wet etch/dry etch process which enabled the removal of the oxide layer from the active-area electrodes.

Our process improvements resulted in an increase in overall yield from roughly 5-10 percent to over 90 percent. In all, ten device chips (containing 36 active sites each) of the submicron-period design were successfully fabricated for Genometrix.

1.2 Microfluidic Biodevices and Applications of Three-Dimensional Laser Processing to Biodevices

Sponsors

Defense Advanced Research Projects Agency
U.S. Air Force - Office of Scientific Research

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1.2.1 Program Summary

This is a collaborative project between the Research Laboratory for Electronics and the Whitehead Institute for Biomedical Research to develop microelectromechanical systems for biochemical analysis (BioMEMS). The main emphasis is on developing microfabricated fluidic devices for sequencing and typing of DNA and protein. In this program, we invent new microfluidic devices and develop new microfabrication methods such as lithography, glass bonding, and molded polymer replication for applications in genetics and cell biology. Our program engineers advanced automation and optical sensors to support BioMEMS development.

Supported by funding from the National Institutes of Health, the Microfluidic Biodevices program installs new methods of high-volume DNA sequencing at Whitehead's Center for Genome Research. Funding from the Defense Advanced Research Projects Agency and the U.S. Air Force's Office of Scientific Research supports advanced applications in Department of Defense forensics and detection of pathological microbes.

1.2.2 Publication

Young, A., and D.J. Ehrlich. "Quantitative Injection of 100-picoliter Samples for Capillary and Microchip Electrophoresis." Submitted to *J. Anal. Chem.*

